

The Functional Significance of Lipid Diversity: Orientation of Cholesterol in Bilayers Is Determined by Lipid Species

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One of the main building blocks of cellular membranes is lipids, which form a two-dimensional fluid matrix and in which membrane-associated proteins are able to carry out their various functions. In 1972, Singer and Nicolson¹ proposed the “fluid mosaic” model to describe the structural features of biological membranes. Although the basic premise of this model (i.e., integral proteins diffusing more or less freely in a two-dimensional viscous phospholipid bilayer solvent) still stands, the plasma membrane has since been shown to be considerably more complex, especially in regard to the diversity and function of lipids.² An example are lipid rafts, regions of membranes enriched in certain types of lipids and cholesterol, which appear to act as platforms for the colocalization of proteins involved in intracellular signaling pathways.³

Cholesterol is found in all animal cell membranes and is required for proper membrane permeability and fluidity. It is also needed for building and maintaining cell membranes, and may act as an antioxidant.⁴ Recently, cholesterol has also been implicated in cell signaling processes, and is suggested to enable lipid raft formation in the plasma membrane.^{5,6} In biological membranes, the lateral sequestration of lipids with polyunsaturated fatty acid (PUFA) chains into membrane domains depleted of cholesterol has been hypothesized to have an important role in neurological function and in alleviating a number of health-related problems.⁷ There seems to be a strong aversion of the disordered polyunsaturated chains to the rigid planar surface of cholesterol, and this is thought to be the major driving force for this kind of domain formation.

Recently, neutron studies of deuterated cholesterol incorporated into 1,2-diarachidonoylphosphatidylcholine (diC20:4PC) bilayers found cholesterol sequestered inside the membrane, in contrast to its usual position with the hydroxyl group located near the lipid/water interface.^{8,9} Complementary experiments using “headgroup”- and “tail”-deuterated cholesterol revealed that the molecule in fact lies flat in the bilayer center.

Molecular dynamics simulations⁷ using the MARTINI coarse-grained force field have shown that the angle of cholesterol with respect to the bilayer normal varies with the number of double bonds present in the lipid fatty acid chains; that is, unsaturation increases the tilt angle. It was also shown that the frequency of cholesterol’s flip-flop between bilayer leaflets dramatically increases with the number of double bonds. Therefore, cholesterol’s ability to flip-flop more rapidly in the presence of PUFA lipids is enticing as a cell signaling mechanism in response to changing conditions in

membrane fluidity or stress. It is therefore also possible that in a mixed bilayer of saturated and polyunsaturated lipids, cholesterol can be made to flip between upright and flat orientations.

Finally, it should be pointed out that cholesterol may regulate membrane fluidity not just by the formation of lateral domains, as is so much the focus of lipid raft research, but also by its wholesale movement across the bilayer, which in turn controls membrane protein function.⁶ It is known that cholesterol’s presence in phospholipid bilayers decreases both their fluidity and permeability,¹⁰ but this is most likely not the case when cholesterol resides in the bilayer center.

We have carried out neutron diffraction experiments to test the hypothesis that cholesterol can revert to its upright position at some critical concentration of monounsaturated 1-palmitoyl-2-oleoylphosphatidylcholine (C16:0-18:1PC, POPC) or disaturated 1,2-dimyristoylphosphatidylcholine (diC14:0PC, DMPC) lipid introduced into diC20:4PC PUFA bilayers. Neutron diffraction data were collected at the Canadian Neutron Beam Centre’s D3 beamline, which is located at the National Research Universal (NRU) reactor (Chalk River, ON), using 2.37 Å wavelength neutrons. The appropriate-wavelength neutrons were selected by the (002) reflection of a pyrolytic graphite monochromator. Samples of oriented multibilayers were prepared using appropriate amounts of PUFA and either POPC or DMPC along with 10 mol % cholesterol. In addition to those with unlabeled cholesterol, samples were also prepared with 10 mol % headgroup-labeled cholesterol (2,2,3,4,4,6-D₆) obtained from C/D/N Isotopes (Pointe-Claire, QB).

The average position of cholesterol molecules within the bilayer was deduced from neutron scattering length density (NSLD) difference profiles, which were obtained via the commonly employed Fourier reconstruction of diffracted intensities measured at various D₂O/H₂O contrasts.¹¹ The 8 mol % D₂O NSLDs from samples with labeled and unlabeled cholesterol were then subtracted to provide the distribution profiles of the deuterium label. This approach takes advantage of the fact that a mixture of 8 mol % D₂O in H₂O has a net zero NSLD, which means that the form factor is independent of the unit cell swelling as a result of hydration.⁸ The uncertainties associated with the difference profiles were obtained from the propagation of standard deviations estimated from repeated diffraction scans while assuming a 95% confidence interval. Finally, data were placed on an absolute NSLD scale by requiring that the area in the NSLD difference profile correspond, within experimental error, to the neutron scattering length difference between six deuterium and six hydrogen atoms.¹⁰ Figure 1 shows a typical result obtained from the procedure described above.

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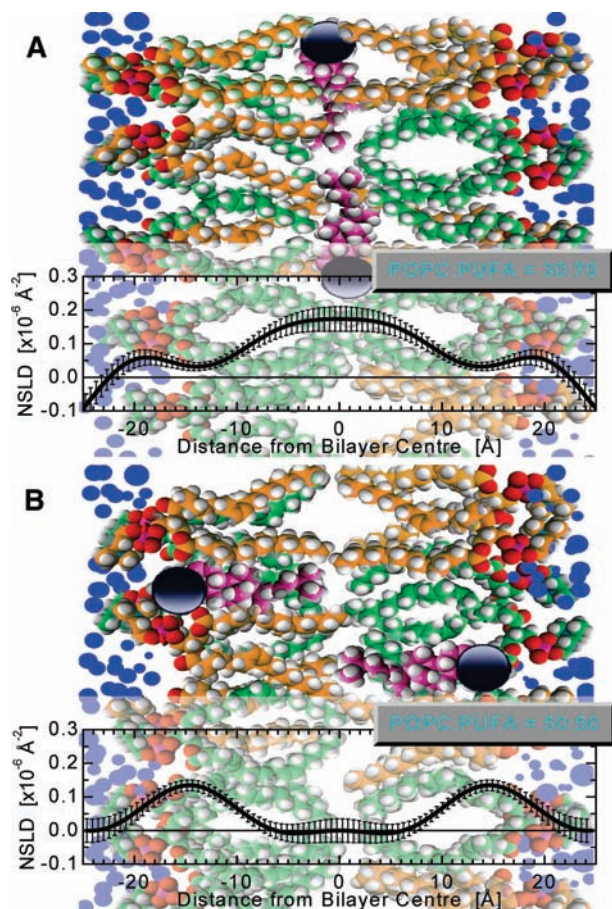


Figure 1. NSLD difference profiles (deuterated minus nondeuterated cholesterol) showing the distribution of cholesterol's deuterium label in (A) 30 and (B) 50 mol % POPC-doped PUFA bilayers. Cartoons depict a schematic view of the various bilayer components, with POPC in yellow, PUFA in green, cholesterol in purple, and deuterium label in black.

Harroun et al.⁹ previously showed that cholesterol is sequestered in the bilayer center of PUFA bilayers. Consistent with this result, we observed the same location for cholesterol in PUFA bilayers doped with small amounts of POPC. Figure 1A shows the NSLD difference profile corresponding to PUFA bilayers containing 30 mol % POPC, in which the cholesterol label was clearly observed in the bilayer center. This was the highest POPC concentration in which cholesterol was unambiguously observed in the bilayer center. However, the situation changed with increasing amounts of POPC. Figure 1B shows the NSLD difference profile for PUFA bilayers containing 50 mol % POPC, the lowest concentration at which we observed cholesterol to revert to its upright orientation. The deuterium label appears to be ~ 15 Å from the bilayer center, placing cholesterol's hydroxyl group within the bilayer's hydrophobic/hydrophilic interfacial region, in excellent agreement with previous data.^{8,13}

The situation when doping PUFA bilayers with DMPC is, however, dramatically different from that with POPC. While 50 mol % POPC was necessary to flip cholesterol into its upright position in PUFA bilayers, Figure 2 shows that only 5 mol % DMPC was necessary to achieve the same effect. This result clearly demonstrates cholesterol's affinity for saturated chains.

It is worth noting that the concentration of DMPC required to flip cholesterol in PUFA bilayers is 2 cholesterol per DMPC.

Interestingly, this corresponds exactly to the maximum solubility of cholesterol in PC bilayers.¹⁴ Since there seems to be an aversion to PUFA–cholesterol interactions,⁷ it also appears that instead of engaging in PUFA–DMPC interactions, all of the DMPC headgroups are being utilized to shield cholesterol from water, thus reducing the energy penalty and enabling cholesterol to revert to its upright orientation. It is also possible to imagine that this may even lead to the formation of nanoscopic domains of DMPC and cholesterol. In contrast, the monounsaturated POPC seems to mix with PUFA lipids, and therefore, it takes at least 5 POPC molecules per cholesterol to induce cholesterol to flip into its upright orientation.

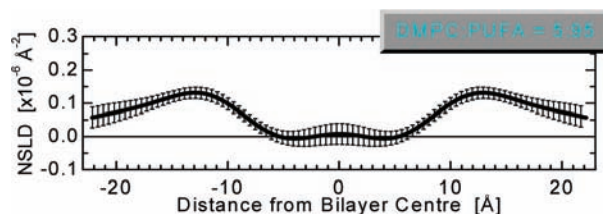


Figure 2. NSLD difference profile showing the distribution of cholesterol's deuterium-labeled headgroup in 5 mol % DMPC-doped PUFA bilayers.

Besides clearly demonstrating the significance of the preference of cholesterol for different lipids, the present results may also be rationalized in terms of what we presently know of biological systems. For example, in plasma membranes, sphingolipids are primarily located in the outer monolayer,¹⁵ whereas unsaturated phospholipids are more abundant in the inner leaflet.¹⁶ Thus, the presence of PUFA in the inner leaflet may serve to enhance the transfer of cholesterol to the outer layer, potentially modifying raft composition and membrane function.

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References

- (1) Singer, S. J.; Nicolson, G. L. *Science* **1972**, *175*, 720.
- (2) Dopico, A. M.; Tigy, G. J. In *Methods in Membrane Lipids*; Dopico, A. M., Ed.; Methods in Molecular Biology, Vol. 400; Humana Press: Totowa, NJ, 2007.
- (3) Calder, P. C.; Yaqoob, P. *J. Nutr.* **2007**, *137*, 545.
- (4) Smith, L. L. *Free Radical Biol. Med.* **1991**, *11*, 47.
- (5) Petrie, R. J.; Schnetkamp, P. P.; Patel, K. D.; Awasthi-Kalia, M.; Deans, J. P. *J. Immunol.* **2000**, *165*, 1220.
- (6) Papanikolaou, A.; Papafotika, A.; Murphy, C.; Papamarcaki, T.; Tsolas, O.; Drab, M.; Kurzchalia, T. V.; Kasper, M.; Christoforidis, S. *J. Biol. Chem.* **2005**, *280*, 26406.
- (7) Marrink, S. J.; de Vries, A. H.; Harroun, T. A.; Katsaras, J.; Wassall, S. R. *J. Am. Chem. Soc.* **2008**, *130*, 10.
- (8) Harroun, T. A.; Katsaras, J.; Wassall, S. R. *Biochemistry* **2006**, *45*, 1227.
- (9) Harroun, T. A.; Katsaras, J.; Wassall, S. R. *Biochemistry* **2008**, *47*, 7090.
- (10) Mathai, J. C.; Tristram-Nagle, S.; Nagle, J. F.; Zeidel, M. L. *Biophys. J.* **2008**, *95*, 69.
- (11) Kučerka, N.; Nieh, M. P.; Pencser, J.; Sachs, J. N.; Katsaras, J. *Gen. Physiol. Biophys.* **2009**, *28*, 117.
- (12) Wiener, M. C.; King, G. I.; White, S. H. *Biophys. J.* **1991**, *60*, 568.
- (13) Kučerka, N.; Perlmutter, J. D.; Pan, J.; Tristram-Nagle, S.; Katsaras, J.; Sachs, J. N. *Biophys. J.* **2008**, *95*, 2792.
- (14) Huang, J.; Buboltz, J. T.; Feigenson, G. W. *Biochim. Biophys. Acta* **1999**, *1417*, 89.
- (15) Brown, D. A.; London, E. *J. Biol. Chem.* **2000**, *275*, 17221.
- (16) Knapp, H. R.; Hullin, F.; Salem, N., Jr. *J. Lipid Res.* **1994**, *35*, 1283.

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